The Expression of Cytokeratins 7, 19, and 20 in Primary and Metastatic Carcinomas of the Liver

Takashi Maeda, M.D., Ph.D., Kiyoshi Kajiyama, M.D., Eisuke Adachi, M.D., Ph.D.,
Kenji Takenaka, M.D., Ph.D., Keizo Sugimachi, M.D., Ph.D., Masazumi Tsuneyoshi, M.D., Ph.D.

Departments of Pathology II (TM, KK, EA, MT) and Surgery II (KT, KS), Faculty of Medicine, Kyushu University, Fukuoka, Japan

We performed immunohistochemical studies on 90 surgically resected liver tumors, including 30 tumors each from hepatocellular carcinoma (HCC), cholangiocarcinoma (CC), and metastatic colorectal adenocarcinoma (MCA), using monoclonal antibodies against cytokeratin (CK) 7, CK 19, and CK 20 to examine the differences in the CK expressions in primary and metastatic carcinomas of the liver. We also investigated the usefulness of such expression in the differential diagnosis in addition to existing markers such as α-fetoprotein, carcinoembryonic antigen, and carbohydrate antigen 19–9. For CK 7, all except for one (97%) of the CCs were diffusely positive, whereas only two (7%) HCCs and one (3%) MCAs were diffusely positive. For CK 19, 23 (77%) CCs and 19 (64%) MCAs were diffusely positive, whereas no HCCs were positive. For CK 20, 22 (74%) MCAs were diffusely positive, whereas no HCC and three (10%) CCs were diffusely positive. The findings concerning the expression of immunohistochemical CK are therefore considered to be useful in addition to the diagnostic criteria when making a differential diagnosis of primary and metastatic carcinomas of the liver.

KEY WORDS: Cholangiocarcinoma, Cytokeratin, Differential diagnosis, Hepatocellular carcinoma, Metastatic carcinoma.

Mod Pathol 1996;9(9):901–909

0893-3952/96/0909-0901$3.00/0 MODERN PATHOLOGY

VOL. 9, NO. 9, P. 901, 1996 Printed in the U.S.A.
Date of acceptance: April 25, 1996.
Address reprint requests to: Masazumi Tsuneyoshi, M.D., Second Department of Pathology, Faculty of Medicine, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812, Japan; fax: 81-92-641-7179.

The differentiation between the primary and metastatic malignant neoplasms of the liver is sometimes difficult or impossible using only light microscopic techniques (1–7). Most cases of hepatocellular carcinoma (HCC) can be diagnosed by histologic analysis, but it is sometimes impossible to differentiate cholangiocarcinoma (CC) from metastatic carcinoma by histologic analysis. Several authors pointed out the usefulness of cytokeratin (CK) immunohistochemical analysis in the differential diagnosis of the liver neoplasms (1–10). Moll *et al.* (11) in 1982 identified and catalogued 19 different CK polypeptides with molecular weights ranging from 40,000 to 70,000 in the human epithelia. Recently, they identified a new CK polypeptide, CK 20, whose expression was almost entirely confined to the gastric and intestinal epithelium, urothelium, and Merkel cells (12). However, the expression of CK 20 in human neoplasms of the liver and its diagnostic utility have not been previously reported. The aim of this study is to examine differences in the expression of CKs 7, 19, and 20 in primary and metastatic carcinomas of the liver, while also evaluating its usefulness in making a differential diagnosis.

MATERIALS AND METHODS

We examined 90 surgically resected liver tumors, consisting of 30 tumors each from HCCs, intrahepatic CCs, and metastatic colorectal adenocarcinomas (MCAs). We fixed the resected liver specimens in 10% formalin and then cut them into 1.0-cm slices. The slice through the maximal diameter of the tumor was divided into blocks, and the blocks were embedded in paraffin and stained with hematoxylin and eosin. The diagnoses of HCC, CC, and MCA were made by routine histologic examination and were based on clinical information and laboratory data. The histologic criteria of HCC were trabecular growth, bile production, eosinophilic cytoplasm, prominent nucleoli, or hyaline bodies. CC was identified by the presence of a small glandular formation composed of small cuboidal cells with

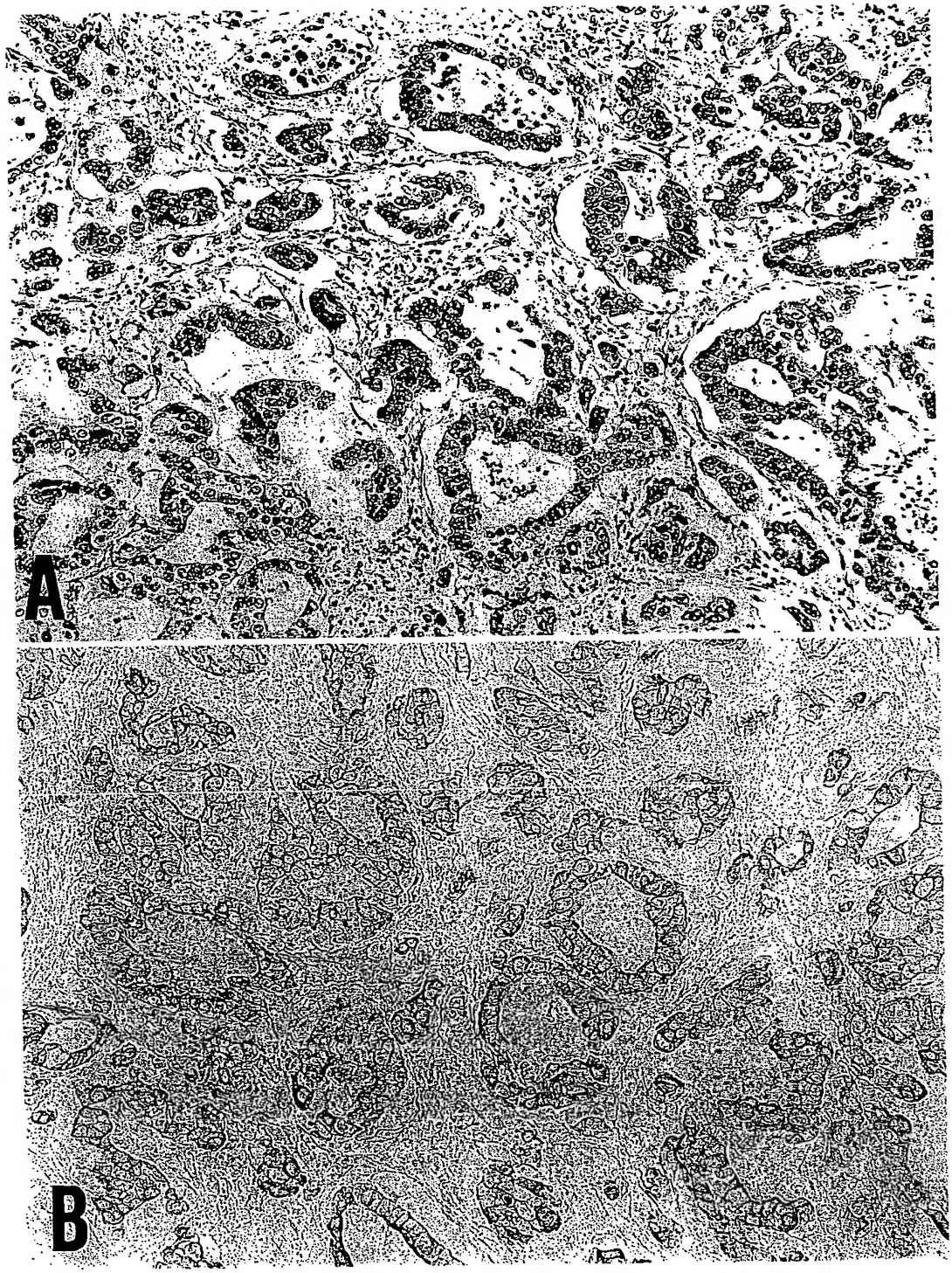

FIGURE 1. A, cholangiocellular carcinoma growing in a tubular fashion (hematoxylin and eosin stain; original magnification, 190×). B, the cytoplasmic membrane of the tumor cells reveals a positive reaction for CK 7, especially at the glandular lumen (original magnification, 190×).

round nuclei resembling a biliary epithelium with abundant fibrous stroma. In MCA cases, the hepatic tumors were identified synchronously or during a follow-up after the operation of the primary site.

Immunohistochemical studies were performed using the avidin-biotin-peroxidase complex technique (13). The primary antibodies used in this study were CK 7, CK 19, CK 20 (monoclonal, DAKO, Glostrup,

TABLE 1 Cytokeratin 7 Expression in Liver Carcinomas

Expression	HCC	CC	MCA
3+	2	29	1
2+	5	1	1
1+	9	0	6
−	14	0	22

HCC, hepatocellular carcinoma; CC, cholangiocarcinoma; MCA, metastatic colorectal adenocarcinoma.

TABLE 2 Cytokeratin 19 Expression in Liver Carcinomas

Expression	HCC	CC	MCA
3+	0	23	19
2+	0	1	2
1+	2	3	4
−	28	3	10

Denmark), α-fetoprotein (AFP) (polyclonal, DAKO), carcinoembryonic antigen (CEA) (monoclonal, Zymed Laboratories, San Francisco, CA), and carbohydrate antigen 19–9 (CA 19–9) (monoclonal, Toray Fuji Bionics, Tokyo, Japan). Sections 4 μm thick were made from 10% formalin-fixed paraffin-embedded materials and then were deparaffinized in xylene and rehydrated in descending dilutions of ethanol. The endogenous peroxidase activity was blocked by incubation with 0.3% hydrogen peroxidase in absolute methanol. The samples were digested with 0.1% trypsin (Type II, T-8003, Sigma, St. Louis, MO) before incubation with the primary antibodies, which included CK 7, CK 19, CK 20, and CA 19–9. Background staining was minimized by preincubation with 1% normal goat or sheep serum for 10 minutes. The sections were incubated with primary antibodies for 90 minutes at room temperature. This was followed by staining with an avidin-biotin-peroxidase kit (Nichirei, Tokyo, Japan). Diaminobenzidine tetrahydrochloride was used as the chromogen. Finally, the sections were counterstained with methyl green. Non-neoplastic bile duct epithelium was used as a positive control for CK 7 and CK 19. Non-neoplastic colonic mucosa was used as a positive control for CK 20. The negative controls consisted of substituting mouse normal serum for the primary antibodies.

Immunopositivity was described as negative (−) when no tumor cells were positive, as sporadically positive (+) when less than 10% of the cells were positive, as focally positive (2+) when 10 to 50% of the cells were positive, and diffusely positive (3+) when more than 50% of the cells were positive.

RESULTS

Histologic Results

The HCCs included 3 well-differentiated, 21 moderately differentiated, and 6 poorly differenti-

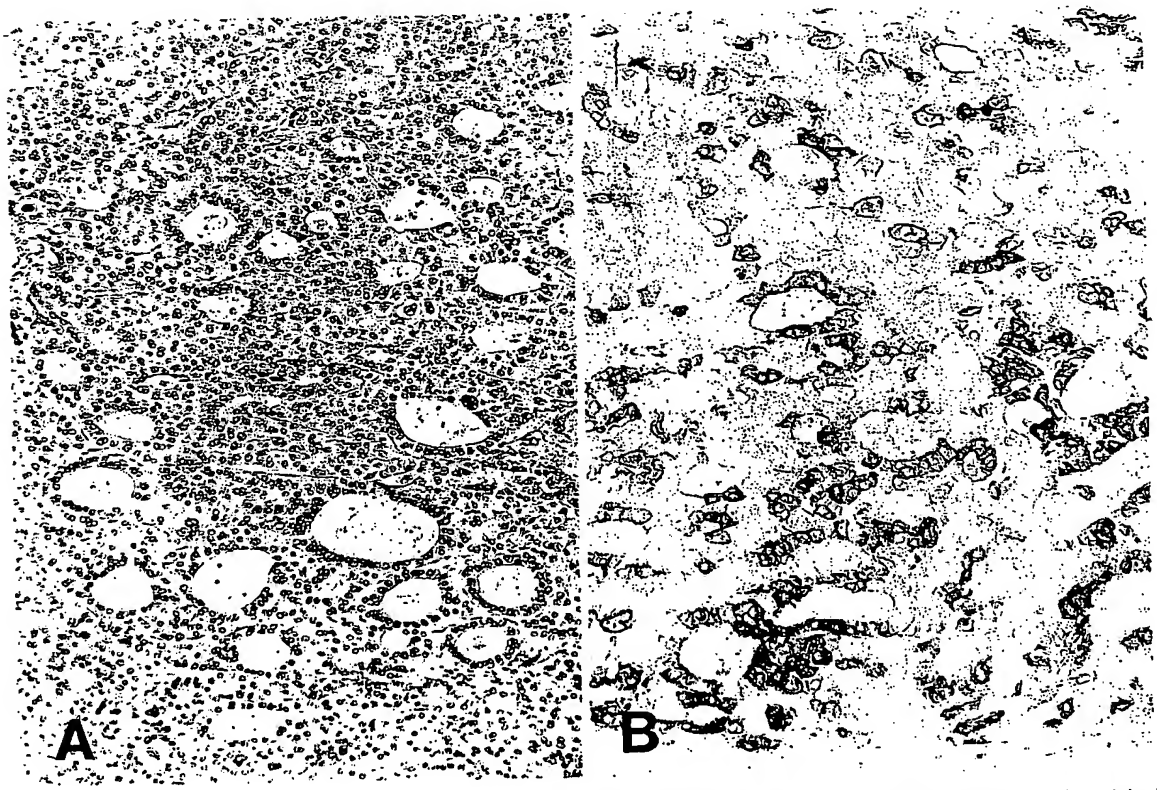

FIGURE 2. A, moderately differentiated HCC growing in a trabecular and pseudoglandular pattern (hematoxylin and eosin stain; original magnification, 108×). B, the tumor cells forming pseudoglandular structures reveal a positive reaction for CK 7 (original magnification, 230×).

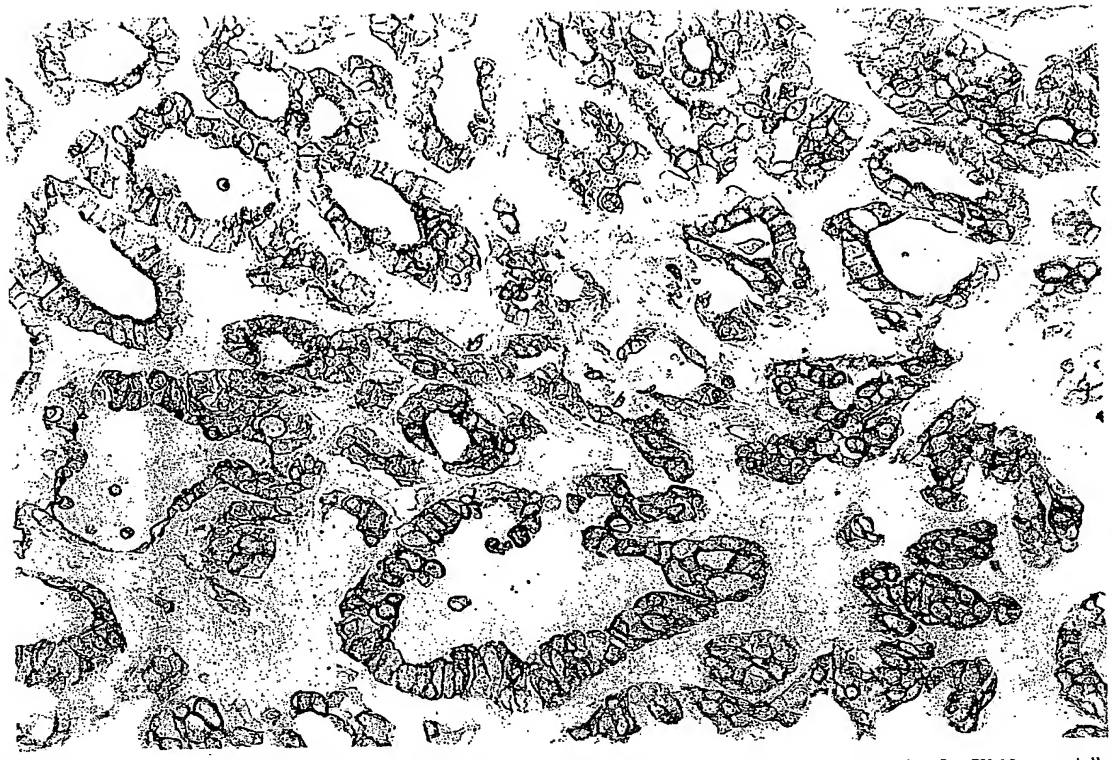

FIGURE 3. The cytoplasmic membrane of the tumor cells of cholangiocellular carcinoma reveals a positive reaction for CK 19, especially at the glandular lumen (original magnification, 190×).

ated HCCs. The CCs included 3 well-differentiated, 21 moderately differentiated, and 6 poorly differentiated CCs. MCAs included 8 well-differentiated, 21 moderately differentiated, and 1 poorly differentiated MCAs. The typical HCC demonstrated a trabecular pattern lined by endothelial cells, intracellular bile droplets, and cytoplasmic hyaline globules, whereas the typical CC showed a tubular pattern with an abundant fibrous stroma, intracellular mucin droplets, fairly uniform nuclei and pale cytoplasm. Although MCA usually resembles CC, the eosinophilic cytoplasm, nuclear palisading, and massive necrosis seemed more conspicuous in MCA than in CC. The fibrous stroma was less prominent in MCA than CC. Most cases of HCC could be diagnosed by histologic examination, whereas it was difficult to differentiate CC from MCA, especially in cases in which the clinical information proved to be insufficient.

Immunohistochemical Results

The results of the immunostaining of the CKs are shown in Tables 1 through 6.

CK 7

All of the CCs except for one (97%) were diffusely positive (Table 1) (Fig. 1), whereas two (7%) HCCs and one (3%) MCA were diffusely positive. Five HCCs and one MCA were focally positive, but less than 30% of the tumor cells demonstrated a positive reaction. The cytoplasm of the tumor cells revealed a positive reaction, especially at the membranous site. In the CK 7-positive HCCs, the tumor cells forming pseudoglandular structures showed a positive reaction (Fig. 2). In addition, the non-neoplastic bile ducts were strongly positive, whereas the surrounding liver parenchyma was negative.

CK 19

Twenty-three (77%) CCs and 19 (64%) MCAs were diffusely positive (Table 2) (Fig. 3), whereas no HCCs were diffusely positive. In 28 (93%) HCCs, no positive tumor cells were found. The cytoplasm of the tumor cells, like CK 7, revealed a positive reaction. The non-neoplastic bile ducts were strongly

TABLE 3 Cytokeratin 20 Expression in Liver Carcinomas

Expression	HCC	CC	MCA
3+	0	3	22
2+	1	4	7
1+	5	5	1
–	24	18	0

HCC, hepatocellular carcinoma; CC, cholangiocarcinoma; MCA, metastatic colorectal adenocarcinoma.

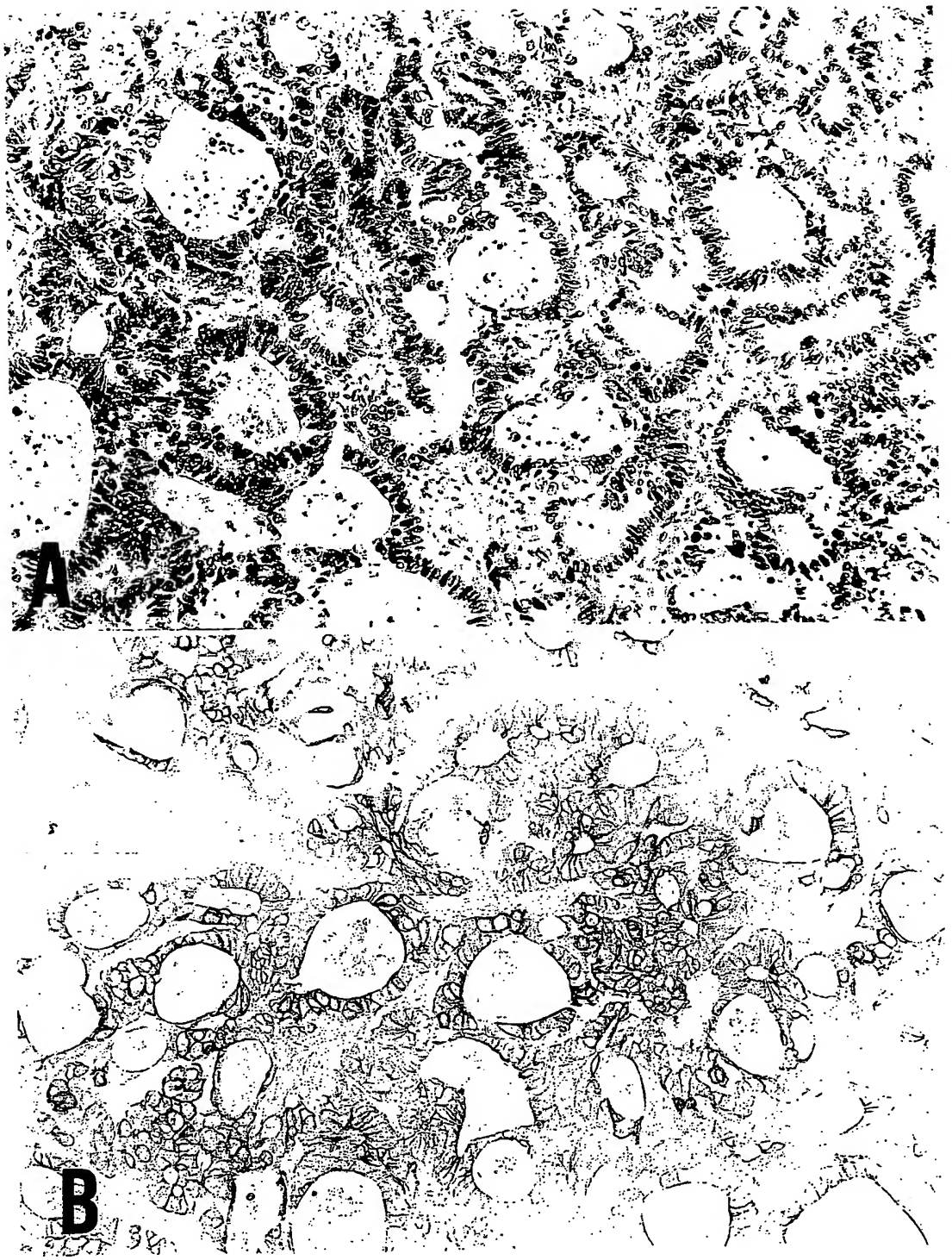

FIGURE 4. A, metastatic adenocarcinoma growing in a tubular fashion (hematoxylin and eosin stain; original magnification, 190×). B, the cytoplasmic membrane of the tumor cells reveals a positive reaction for CK 20, especially at the glandular lumen (original magnification, 180×).

positive, but the surrounding liver parenchyma was negative.

CK 20

Twenty-two (74%) MCAs were diffusely positive (Table 3) (Fig. 4), whereas no HCC and three (10%) CCs were diffusely positive. The cytoplasm of the tumor cells, like CK 7 and CK 19, revealed a positive reaction. In CK 20-positive HCC, the tumor cells forming pseudoglandular structures revealed a positive reaction (Fig. 5). Some multinucleated giant cells in one of the poorly differentiated HCCs also

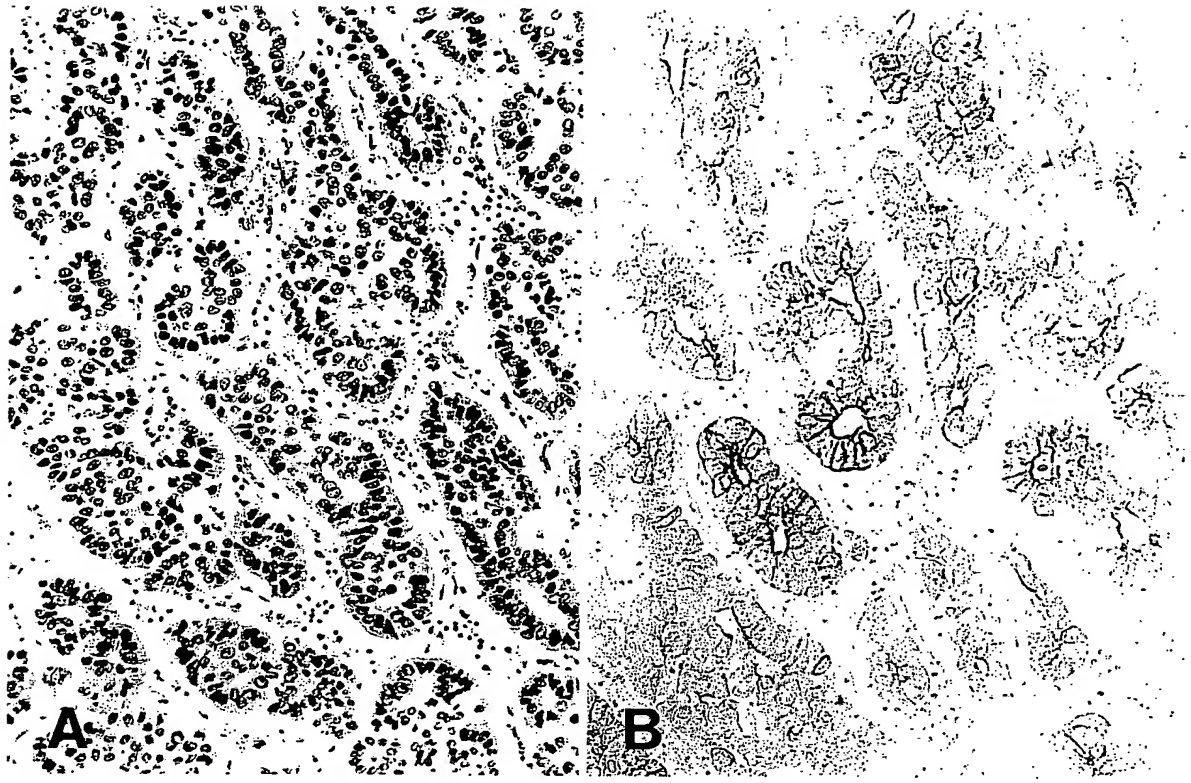

FIGURE 5. A, moderately differentiated HCC growing in a trabecular and pseudoglandular pattern (hematoxylin and eosin stain; original magnification, 190×). B, the tumor cells forming pseudoglandular structures reveal a positive reaction for CK 20 (original magnification, 240×).

revealed a positive reaction for CK 20. Neither the non-neoplastic bile ducts nor the surrounding liver parenchyma was positive. In CK 20-positive CCs, the tumor cells around the CK 20-negative, non-neoplastic bile ducts revealed a positive reaction (Fig. 6). The transition between carcinoma cells and the bile duct epithelium in the portal tracts either within or near the tumor was observed not only in CCs but also in MCAs. In these MCA lesions, the tumor cells were negative for CK 7 and 19 but positive for CK 20, whereas the bile duct epithelium was positive for CK 7 and 19 but negative for CK 20 (Fig. 7).

AFP

Three (10%) HCCs were diffusely positive, whereas 2 (7%) CCs and no MCAs were diffusely positive (Table 4). In 19 (64%) HCCs, 25 (83%) CCs, and 28 (93%) MCAs, no positive tumor cells were found.

CEA

Twenty-six (87%) MCAs were diffusely positive, whereas 13 (43%) CCs and no HCCs were diffusely positive (Table 5). In all of the HCCs, no positive tumor cells were found.

CA 19–9

Nineteen (63%) CCs and 15 (50%) MCAs were diffusely positive, whereas all of the HCCs were negative (Table 6).

DISCUSSION

Differentiating between primary and metastatic malignant neoplasms of the liver is sometimes difficult or even impossible by means of conventional histologic staining. Several authors investigated the use of immunohistochemical analysis in the differentiation of liver carcinomas using various antibodies (14–24). Hurlimann and Gardiol (6) examined various antibodies, including CEA, CA 19–9, and CK 19, for a differential diagnosis of liver carcinomas and concluded that it was impossible immunohistochemically to differentiate CC from most metastatic carcinomas. In our study, AFP, CEA, and CA 19–9 were also considered to be somewhat useful markers for differentiating HCC from CC and MCA, although the differentiation between CC and MCA was difficult.

Recently, using various monoclonal anti-CK antibodies, some investigators were able to distinguish most HCCs from CCs or metastatic carcino-

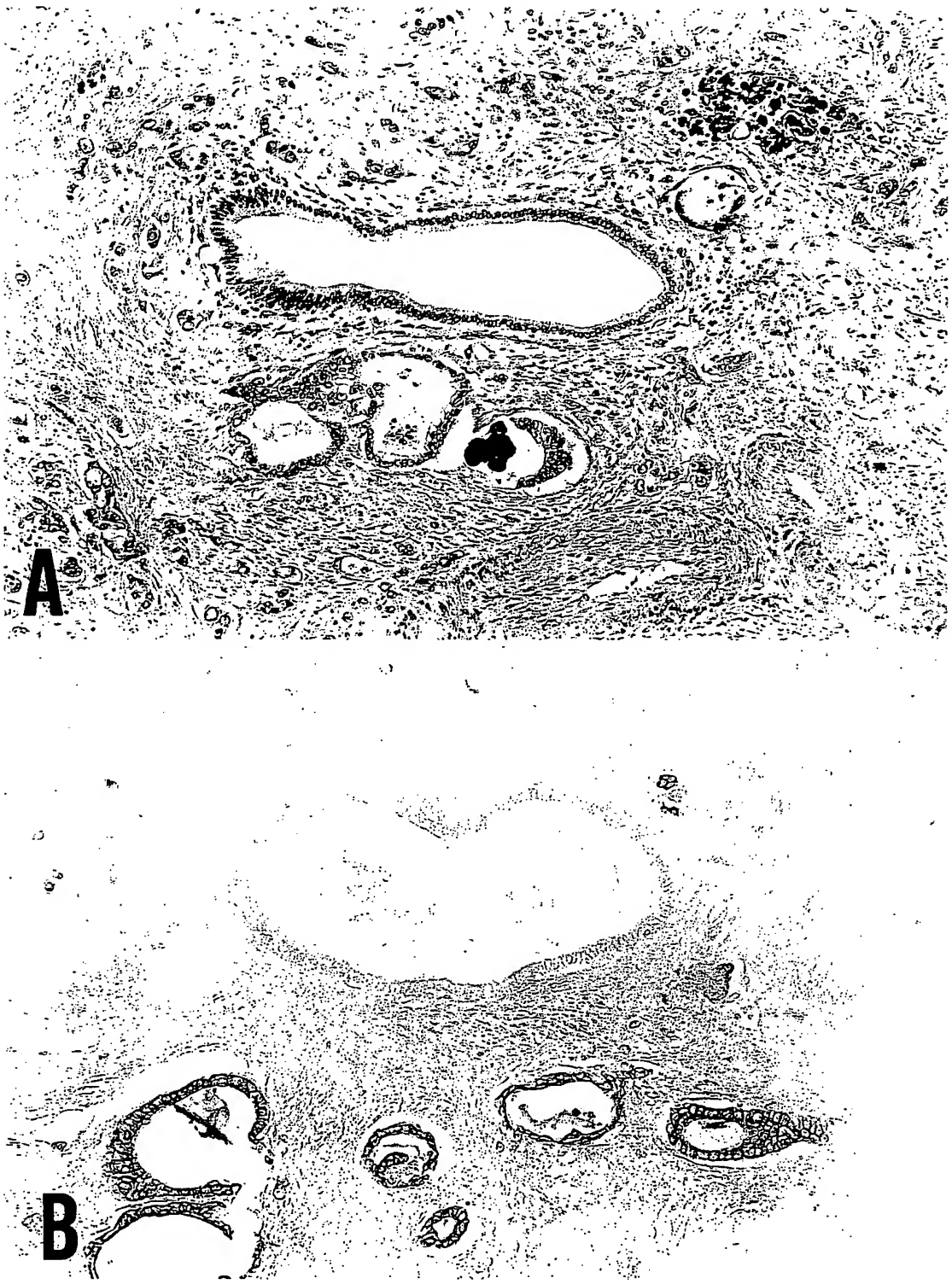

FIGURE 6. A, cholangiocellular carcinoma infiltrates around the non-neoplastic bile ducts (hematoxylin and eosin stain; original magnification, 138×). B, only tumor cells around the non-neoplastic bile ducts reveal a positive reaction for CK 20 (original magnification, 136×).

mas (1–10). The intermediate filament family consists of five subclasses: vimentin, desmin, glial fibrillary acidic protein, neurofilaments, and CKs (25). Neoplastic cells usually retain the intermediate filament type of their cell of origin and antibodies directed against the intermediate filament subclasses are currently being used in the differential diagnosis of human neoplasms.

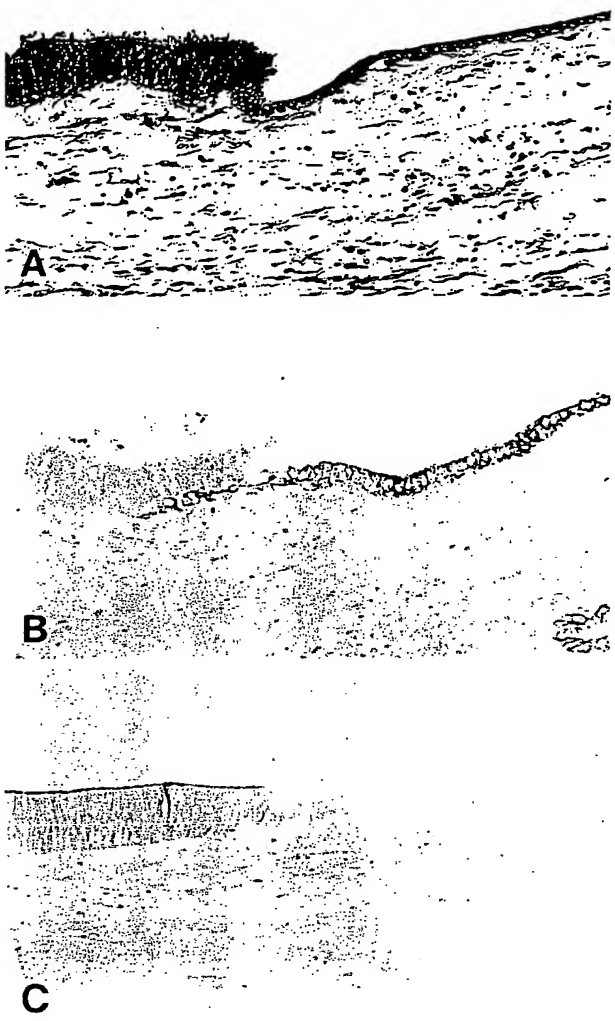

FIGURE 7. A, the tumor cells of MCAs replace the bile duct epithelium in the portal tract (hematoxylin and eosin stain; original magnification, 210×). B, the tumor cells reveal a negative reaction for CK 7, whereas the bile duct epithelium is positive (original magnification, 210×). C, the tumor cells reveal a positive reaction for CK 20, whereas the bile duct epithelium is negative (original magnification, 210×).

Some authors reported that HCC was immunoreactive to CK 8 and CK 18 but generally not to CK 7 or CK 19. In contrast, CC was immunoreactive to CK 7, CK 8, CK 18, and CK 19 (1, 2, 6, 8–10). The expression of CK 7 in metastatic carcinoma of the liver has only rarely been reported (1, 2). Balaton *et al.* (2) reported that all of the metastatic adenocarcinomas that they studied were positive for CK 7, except for colorectal adenocarcinomas and a carcinoid tumor. Osborn *et al.* (7) reported that the CK 7 antibody seems able to distinguish carcinomas originating in the pancreatic ducts from, for instance, those originating in the stomach or large bowel. In this study, the positivity of the expression of CK 7 in CCs was significantly higher

TABLE 4. α-Fetoptotein Expression in Liver Carcinomas

Expression	HCC	CC	MCA
3+	3	2	0
2+	4	1	0
1+	4	2	2
−	19	25	28

HCC, hepatocellular carcinoma; CC, cholangiocarcinoma; MCA, metastatic colorectal adenocarcinoma.

TABLE 5. Carcinoembryonic Antigen Expression in Liver Carcinomas

Expression	HCC	CC	MCA
3+	0	13	26
2+	0	5	4
1+	0	6	0
−	30	6	0

HCC, hepatocellular carcinoma; CC, cholangiocarcinoma; MCA, metastatic colorectal adenocarcinoma.

TABLE 6. Carbohydrate Antigen 19-9 Expression in Liver Carcinomas

Expression	HCC	CC	MCA
3+	0	19	15
2+	0	4	7
1+	0	5	4
−	30	2	4

HCC, hepatocellular carcinoma; CC, cholangiocarcinoma; MCA, metastatic colorectal adenocarcinoma.

than that in HCCs or MCAs; it is therefore considered to be useful in the differential diagnosis of CCs from HCCs and MCAs. The positivity of the CK 19 expression of CCs and MCAs was significantly higher than that of HCCs; it is therefore considered to be useful in the differential diagnosis of HCCs from CCs and MCAs.

CK 20, which is a newly recognized member of the CK family, can be found in the gastric foveolar cells and intestinal absorptive cells (12). Few studies on the CK 20 expression of neoplasms have been reported (26–28). In this study, the positivities of the CK 20 expression in MCAs was significantly higher than those in HCCs and CCs; it thought to be useful in the differential diagnosis of MCAs from HCCs and CCs.

The transition between carcinoma cells and the bile duct epithelium in the portal tracts either within or nearby the tumor has been considered to be one of the most important factors in accurately diagnosing CC. Weinbren and Mutum (29) analyzed the pathologic characteristics of CC and stated that growth along the duct wall was a common finding in most CCs, whereas this formation was not noted in cases of metastatic adenocarcinoma. Nakajima *et al.* (30) mentioned that tumor cells were seen growing along the bile duct lumen and replacing the benign epithelium in 11 (12%) of 89 cases of CCs. However, this appearance was seen not only in CCs but also in MCAs in our study. In these lesions,

MCA cells were negative for CK 7 and 19 but positive for CK 20, whereas the bile duct epithelium was positive for CK 7 and 19 but negative for CK 20. We therefore consider that MCAs also involve the bile ducts and seem to replace these epithelia in a manner similar to that of CCs.

In addition, some HCC cells showing a pseudoglandular structure revealed a positive reaction for CK 7 or CK 20, which suggested a correlation between CK expression and glandular formation. The CK pattern is thought to be essentially preserved during neoplastic transformation (11), and we essentially agree with this hypothesis. However, some CCs demonstrated a positive reaction for CK 20, whereas all non-neoplastic bile ducts and ductules were negative. This suggested a correlation between the CK 20 expression and neoplastic transformation of the bile ducts in some CCs.

In conclusion, CK 7, 19, and 20 expression in HCCs, CCs, and MCAs were found to be significantly different from each other. Therefore, the immunohistochemical CK expressions are thought to be useful in helping to make a differential diagnosis between primary and metastatic carcinomas of the liver in addition to other existing markers, such as AFP, CEA, and CA 19–9.

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